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Molecular landscape of muscle-invasive bladder cancer: Patient age matter?

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Dear editor, muscle-invasive urothelial bladder cancer (MIBC) is diagnosed in patients at a median age of approximately 70 years and is therefore, predominantly a disease of the elderly. This is likely due to an age-related accumulation of exposure to carcinogens such as tobacco smoke and occupational toxins, which are the main risk factors for this disease. Although the relative rate of non-muscle invasive bladder cancer is higher at earlier ages, younger patients can also acquire MIBC. Little is known about age-related differences in the molecule landscape MIBC.

Recently, several groups including The Cancer Genome Atlas (TCGA) have conducted a comprehensive molecular characterization of MIBC (1), and have included patients across a

wide age spectrum. These analyses demonstrated the mutational landscape and that MIBC can be classified into distinct molecular subtypes based on genomic and transcriptomic alterations. The aim of our study was to investigate molecular differences in MIBC in younger compared to older patients. Since younger patients have had less time to accumulate mutations, we hypothesized that the total mutational burden (TMB) would be lower in younger patients. To address this question, we analyzed the TCGA bladder cancer cohort, in which the median age was 69 years (range 34 – 90 years) and 22/412 patients (5.3%) were below the age of 50 years. Of note, smoking history and number of pack years were not associated with patient age in this cohort.

Our analysis revealed that the somatic mutation rate of in MIBC is associated with patient age. When the patient cohort was stratified into quartiles based on TMB, the median age in the 1st quartile was 65 years, compared to 70 years in the other three quartiles (**Figure 1A**). MIBC patients with a lower (1st quartile) neoantigen load were also younger (65 years) than patients with a higher (2nd-4th quartiles) neoantigen load (69 years, $p<0.001$) (**Figure 1B**). Patients with wild type RB1 and TP53, key tumor suppressors in MIBC, were not significantly younger than patients with genomic alterations in these two genes (**Figure 1C**). However, the MIBC in all 36 patients below 54 years appeared to harbor wild type RB1, as measured by TCGA whole exome sequencing.

Transcriptomic profiles were also found to be related to patient age. We determined the molecular subtype using the consensus classifier from The Bladder Cancer Molecular Taxonomy Group (2) and observed that the prevalence of subtypes differed by age (**Figure 1D**). The age of patients with luminal papillary tumors (64 years) was significantly lower than patients with other subtypes (70 years, $p<0.001$). Other datasets of invasive bladder cancer confirmed this finding (3, 4) patients with luminal papillary tumors were younger when compared to patients with other subtypes (**Figure 1E**).

There was evidence for less immune-related signaling in MIBC of younger patients. Expression of genes found on the surface of lymphocytes (e.g. CD19, CD79A) was significantly lower in MIBC of patients ≤ 50 years of age (**Figure 1F**). We observed that gene expression signatures for IFN-y and chemokine signalling were downregulated in patients ≤ 50 years of age (**Figure 1G**, $p<0.04$). Tumor-infiltrating lymphocytes assessed by pathological examination were absent in 11/22 (50%) MIBC in patients ≤ 50 years of age. This rate was lower in patient > 50 years of age (149/382, 39%), but this difference did not reach statistical significance (**Figure**

1H, p=0.4). These findings are consistent with the higher proportion of luminal papillary tumors in younger patients.

Age dependent differences of the molecular landscape have also been reported in other cancers (5). Similarly, we identified a pattern of age-dependent alterations in the molecular landscape of MIBC in the TCGA dataset. MIBC in patients ≤ 50 years of age showed a lower total mutational burden, a lower neoantigen load, a higher rate of luminal papillary subtype and less intratumoral immune signaling. Fewer background somatic mutations at a younger age may be due to less exposure to carcinogens, more limited field cancerization of the urothelium and less time for exposure to aberrant APOBEC activity. It is plausible that the development of MIBC at a young age may be less reliant on the steady accumulation of many genomic alterations. Instead a combination of potent alterations in a few key driver genes or a specific predisposition based on germline alterations may cause bladder cancer at a younger age. Our findings suggest that a molecular characterization of MIBC in a larger cohort of young patients may reveal new insights into the evolution of MIBC.

A low background of somatic mutations may result in less immunogenic tumors. Moreover, all of our identified age-related parameters have been reported as unfavorable predictors of response to checkpoint inhibition (6). However, the hypothesis that MIBC in younger patients is less responsive to checkpoint inhibition needs to be investigated in clinical trials.

Conflicts of interest

None disclosed

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Figure Legend

- **A)** Boxplot indicating total mutational rate in somatic mutations per megabase grouped into quartiles and analyzed in relation to patient age. Cancers with low total mutational burden (1st quartile) were more frequently found in younger patients.
- **B)** Boxplot indicating neoantigen load grouped into quartiles and analyzed dependent on patient age. A low neoantigen load was associated with younger patient age.
- **C)** Gene status (somatic mutations) of RB1 and TP53 dependent on patient age. Patient with wild type in RB1 were younger than patients with a somatic mutation (p=0.06). All cancers of a patient below the age of 54 years had a wild type gene status in RB1. WT = Wild type, Mut = Mutated.
- **D)** Boxplot indicating differences of patient age dependent on molecular subtypes. Patients with luminal papillary tumors were younger than patients with other subtypes. Molecular subtypes were determined using the classifier from The Bladder Cancer Molecular Taxonomy Group.
- **E)** Boxplot indicating differences of patient age dependent on molecular subtypes. Similarly, in these two other datasets (Lund GSE32894 and MDA GSE48075), patients with luminal papillary tumors were younger thatn patients with other subtypes.
- **F)** Volcano plot of gene expression analysis between patients ≤ 50 years compared to patients > 50 years. Underexpressed genes in young patients were associated with lymphocyte surface markers.
- **G)** Boxplots indicating IFN-y and chemokine signaling in patients ≤ 50 years compared to patients > 50 years of age. Signature scores for both immune signalling mechanisms were significantly lower in patients ≤ 50 years of age.
- **H)** Tumor infiltrating lymphocytes assessed by pathological examination were quantified by central pathological review form the TCGA consortium. Absence of infiltrating lymphocytes was more frequently in cancers of patients ≤ 50 years when compared to patients > 50 years (p=0.4).